Variation in DNA Mixture Interpretation:
Observations from a NIST Interlaboratory Study

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AAFS Annual Meeting – Criminalistics Session
February 19, 2015

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Past and Present Funding: Interagency Agreement between the National Institute of Justice and NIST Office of Law Enforcement Standards

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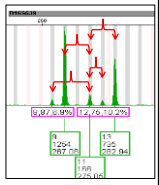
Challenging Mixtures - Uncertainty

- **If allele dropout is a possibility** (e.g., in a partial profile), then there is uncertainty in whether or not an allele is present in the sample…and therefore what genotype combinations are possible
- **If different allele combinations are possible** in a mixture, then there is uncertainty in the genotype combinations that are possible…

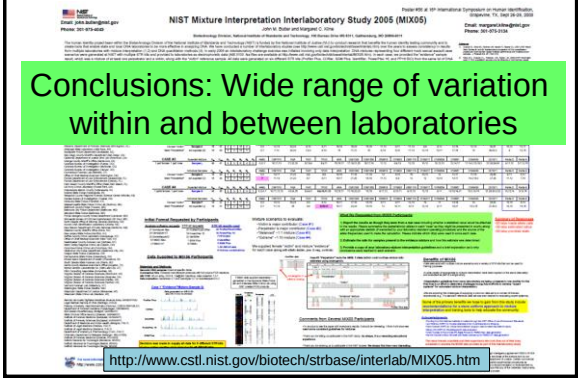


Possible allele pairing with the 11

Previous Interlaboratory Studies

- MSS 1 (1997) – 22 labs participated
- MSS 2 (1999) – 45 labs participated
- MSS 3 (2000-2001) – 74 labs participated
- MIX05 (2005) – 69 labs participated

MIX05 Poster Presentation at ISHI


NIST Mixture Interpretation Interlaboratory Study 2005 (MIX05)

Conclusions: Wide range of variation within and between laboratories

http://www.cstl.nist.gov/biotech/strbase/interlab/MIX05.htm

How MIX13 differs from MIX05 study

	MIX13 (2013)	MIX05 (2005)
Response	108 labs	69 labs
Number of cases provided	5 cases	4 cases
Case types being mimicked	Sexual assault & touch evidence	Sexual assault evidence
Mixture complexity	2, 3, >3-person (potentially related, low-template, inclusion/exclusion)	all 2-person (all unrelated, male/female; various major/minor ratios)
Scenarios provided	Yes	No

MIX 13 – NIST Interlaboratory Study on Mixture Interpretation - **Purpose**

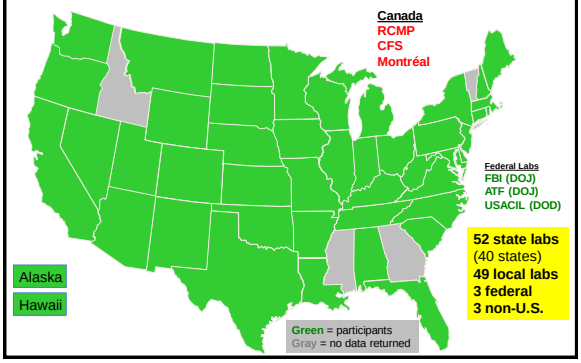
- MIX05 – conducted in 2005. Since then a great deal of effort has been focused on improvements in DNA mixture interpretation.
- 2010 SWGDAM Guidelines approved in January 2010 – many labs have changed their protocols recently.
- MIX13 – Interpretation challenge – no samples to run.

MIX 13 – NIST Interlaboratory Study on Mixture Interpretation - **Goals**

- (1) To evaluate the current "lay of the land" regarding STR mixture interpretation across the community.
- (2) To measure consistency in mixture interpretation across the U.S. after the publication of the 2010 SWGDAM guidelines.
- (3) To learn where future training and research could help improve mixture interpretation and reporting.

MIX13 Participants from **108 Laboratories**

46 states had at least one lab participate



Purpose of MIX13 Cases

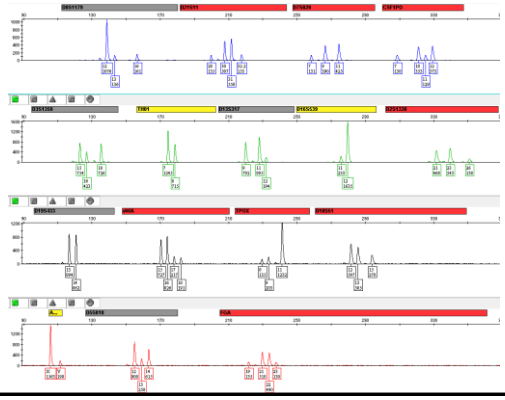
	Challenge provided to study responses
Case 1	~1:1 mixture (2-person)
Case 2	**Low template** profile with potential dropout (3-person)
Case 3	Potential **relative** involved (3-person)
Case 4	Minor component (2-person)
Case 5	Complex mixture (>3-person) with # **of contributors**; inclusion/exclusion issues

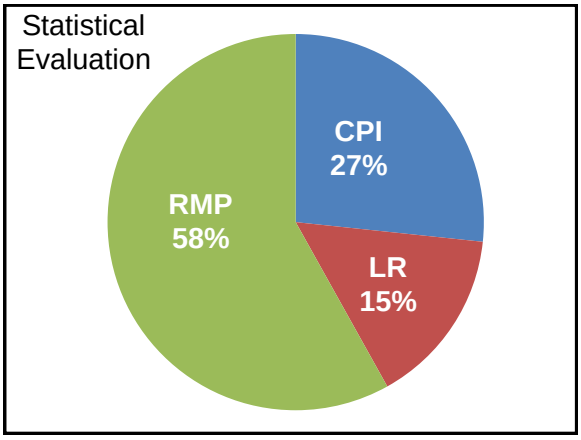
According to German Stain Commission (2009) mixture types: 1 = A, 2 = C, 3 = ?, 4 = B, 5 = ?

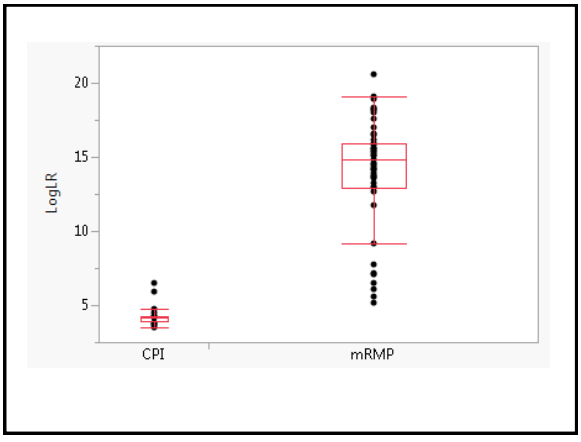
MIX13 Study (Case 04)

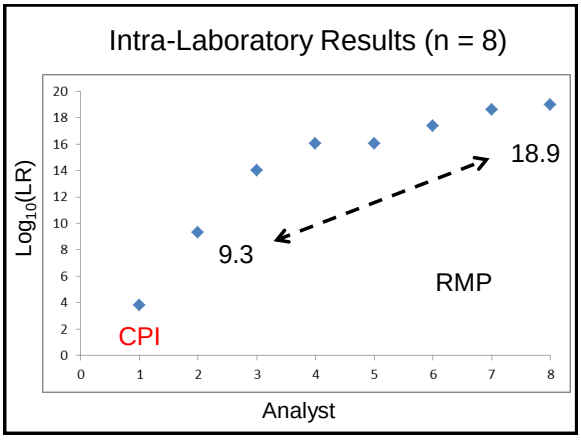
- Summary – Mock sexual assault, 2 person **3.5**:**1** mixture, minor component has alleles below the ST of 150 (required by all labs!)
- Purpose – How many labs would attempt to separate the two components?
- With all labs using the AT/ST – how much variation is expected?

Case 04 – IDPlus




Statistical Evaluation
CPI 27%
LR 15%
RMP 58%

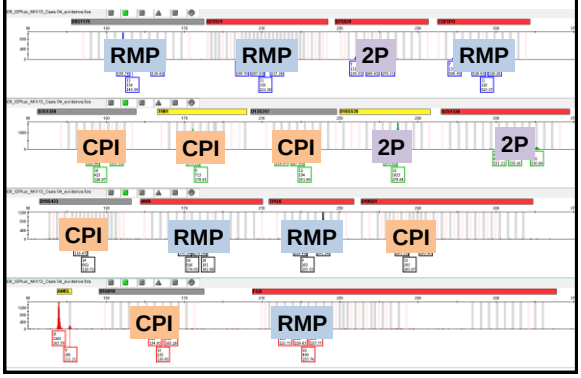

LogLR
20
15
10
5
CPI
mRMP


Intra-Laboratory Results (n = 8)
Log10(LR)
Analyst
18.9
9.3
RMP
CPI

Concerns raised with MIX13

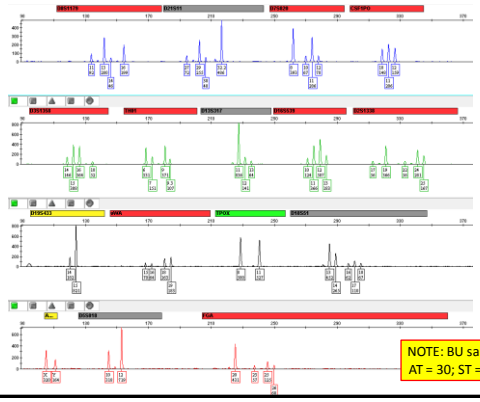
- Labs using RMP, LR – all over the place
- 4.6.2. It is not appropriate to calculate a composite statistic using multiple formulae for a multi-locus profile. For example, the CPI and RMP cannot be multiplied across loci in the statistical analysis of an individual DNA profile because they rely upon different fundamental assumptions about the number of contributors to the mixture.

One Lab's Interpretation



Case 02 – More Complexity

Case 02 – IDFiler



Case 02 – Four Suspects

	Individual	Included?	Ratio
212 pg	Suspect A	Yes	6
53 pg	Suspect B	Yes	1.5
35 pg	Suspect C	Yes	1
	Suspect D	No	--

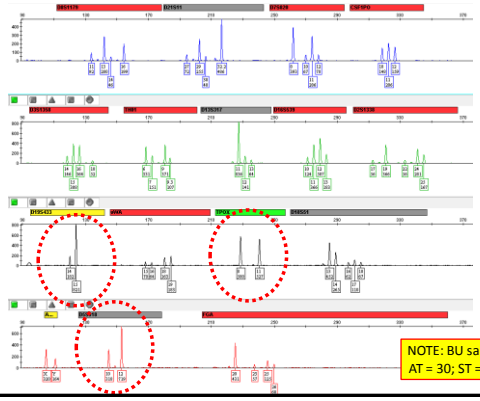
Drop Out Is Possible

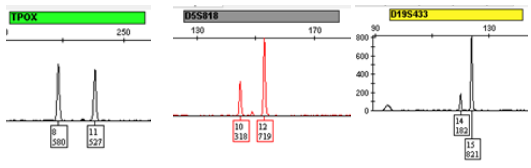
Total Input DNA = 300 pg

Primary Goals

- Primary purpose – is this mixture too complex for interpretation due to the potential of drop-out?
- Several labs – CPI for Suspects A, B and C using a limited number of loci.

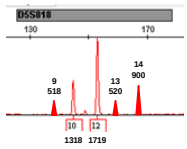
Case 02 – IDFiler





~ 1 in 35

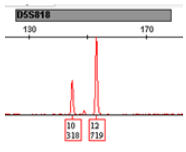
A cautionary note on using CPI when drop-out is possible



$PI = (f_9 + f_{10} + f_{12} + f_{13} + f_{14})^2$

$PI = (0.049+0.051+0.361+0.384+0.141)^2$

PI = 0.986 or **1 in 1.01**



$PI = (f_{10} + f_{12})^2$

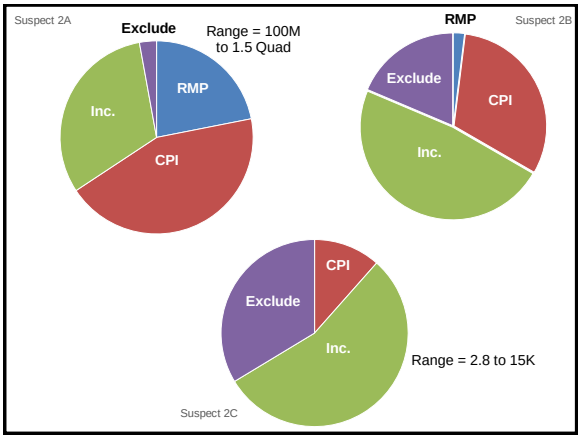
$PI = (0.051 + 0.361)^2$

PI = 0.169 or **1 in 5.92**

Drop-out ***inflates*** your statistics for CPI (not conservative!)

Primary Goals

- Primary purpose – is this mixture too complex for interpretation due to the potential of drop-out?
- Several labs – CPI for Suspects A, B and C using a limited number of loci.
- One lab has included Suspect D (Not in the mixture).



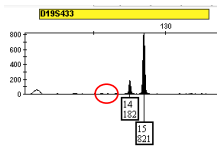
Intra-Laboratory Results (n = 8)

Analyst	Suspect A	Suspect B	Suspect C	Suspect D
1	Inconclusive - A, B, C			Excluded
2	6.74 Quad	23.6	Excluded	Excluded
3	Inconclusive - A, B, C			Excluded
4	9.4 for A, B, C			Excluded
5	4.1 Quint	37	Excluded	Excluded
6	230 for A, B		Inconclusive	Excluded
7	9.4 for A, B		Excluded	Excluded
8	37.3 for A, B		Excluded	Excluded

Concerns raised with MIX13

- D19S433

Contributors
A = 15, 15
B = 14, 15
C = **12**, 14



15 of 108 labs used CPI to include Suspect C (13.8%)

4 of these 15 (26.6%) used D19 as a locus for CPI

A way forward?

Handling Complex Mixtures

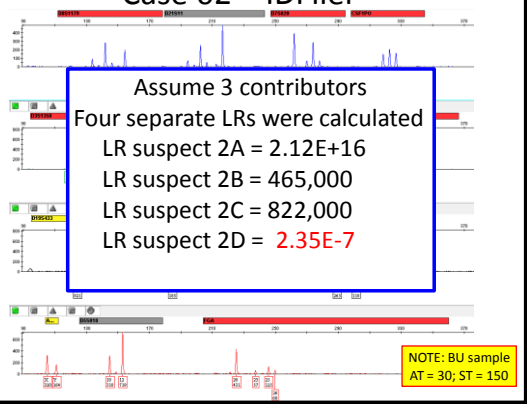
- Stochastic thresholds are necessary in combination with CPI statistics
  - but a stochastic threshold may not hold much meaning for >2 person mixtures (due to potential allele sharing)
- Most labs are not adequately equipped to cope with complex mixtures
  - Extrapolating validation studies from simple mixtures will not be enough to create appropriate interpretation SOPs

David Balding (UK professor of statistical genetics): "LTDNA cases are coming to court **with limited abilities for sound interpretation**." (Rome, April 2012 meeting)

Probabilistic Approaches

- Semi-Continuous (discrete) or Fully-Continuous methods to mixture interpretation provide a way to handle uncertainty in complex profiles (where allelic drop-out is possible).
- These approaches DO NOT use stochastic thresholds and report the significance of an evidentiary match as a Likelihood Ratio (LR).
- These approaches MAY be useful for improving consistency within and between labs.

Case 02 – IDFiler

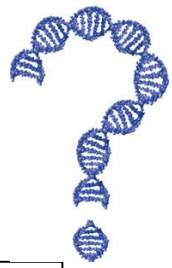


Acknowledgments

National Institute of Justice and NIST Law Enforcement Standards Office

Applied Genetics Group
Dr. Peter Vallone
Becky Hill
Margaret Kline

Dr. Charlotte Word
Dr. Robin Cotton
Dr. Catherine Grgicak



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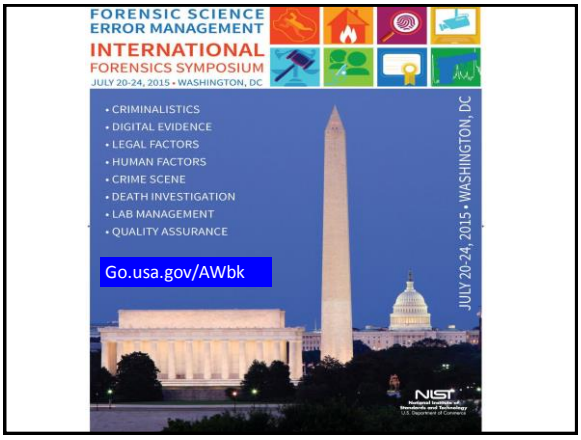
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